PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 27.83814	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/04794	International filing date *(day/month/year)* 29.10.2003	Priority date *(day/month/year)* 29.10.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant FU, Guoliang		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1-9 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28.05.2004	15.02.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Reuter, U Telephone No. +31 70 340-1036

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/04794

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-46 as originally filed

Claims, Numbers

1-55 filed with telefax on 16.12.2004

Drawings, Sheets

1-19 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☒ the claims, Nos.: 56,57

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3,4,10-23,26-32,36,38-44,46,47,49,50,53
	No:	Claims	1,2,5-9,24-25,33-35,37,45,48,51,52,54 and 55
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-55
Industrial applicability (IA)	Yes:	Claims	1-55
	No:	Claims	-

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 The following **documents** are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: US-A-5 744 308 (CLEUZIAT PHILIPPE ET AL) 28 April 1998 (1998-04-28)

D2: TODD ALISON V ET AL: "DzyNA-PCR: Use of DNAzymes to detect and quantify nucleic acid sequences in a real time fluorescent format" CLINICAL CHEMISTRY, AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY. WINSTON, US, vol. 46, no. 5, May 2000 (2000-05), pages 625-630, XP002245984 ISSN: 0009-9147

D3: WO 91/04340 A (CAMBRIDGE BIOTECH CORP) 4 April 1991 (1991-04-04)

2 **NOVELTY** (Art. 33(2) PCT)

2.1 D1 discloses DNA-RNA chimeric probes that are used in an amplification method. The probes comprise a single strand of a RNA polymerase promoter (template portion), a DNA part that can hybridise to the target and leads to the RNAseH cleavage of said target (enzyme acting and target complementary portion) and a RNA part hybridising to the target that can be blocked at it 3' end (target complementary portion) (s. fig. 1, col. 8 l. 37-65, col. 11 first paragraph). The promoter sequence that is comprised in said template portion template portion can be a T7 promoter sequence (col.11 l. 61-67). The T7 promoter sequence, as can be seen on page 43 of D3 comprises two identical sequences (ATA/ATA) that are separated by at least one enzyme acting portion: CTC is the recognition site of the restriction endonuclease Hind I. D1 thus discloses probes containing all the technical features of claim 1.

2.2 D1 furthermore discloses that the DNA-RNA chimeric probe is hybridised to the RNA target. The hybridised target is partially digested by RNAseH to allow the target molecule to be extended by DNA polymerase to complete the probe promoter. RNA polymerase uses the activated promoter to produce single stranded products (fig. 1).

A similar downstream probe is used, the corresponding steps are performed and the single stranded product is again extended by the first probe to complete a cyclic reaction (col. 9).

2.3 The document D1 thus contains all the technical features of the probes claimed in independent claim 1 and dependent claims 2,5-9,24-25.

2.4 Furthermore document D1 discloses a method of detecting a target nucleic acid (first column). The method comprises the steps of contacting probes as disclosed in claim 1 (s. above) with a target and allowing their target complementary portion to hybridize to the target (fig. 1, col. 11), wherein the enzyme acting portion of said probe is at least partially functional (e.g. the hybridizing part that allows RNAseH activity (fig. 1, col. 11). Furthermore the method of D1 comprises the step of creating active double stranded and fully functional promoters ("enzyme acting portions", fig. 1, col. 11). The promoter activity leads to the formation of single stranded nucleic acids ("end products", fig. 1). These transcripts are again annealed to free probes and the promoter portions of said probes are rendered double stranded and fully functional (col. 13 l. 23-52). Repeating these steps (fig. 1) leads to the production of multiple copies of a single stranded nucleic acid that are detected (example 6). This detection implies an indirect detection of other reaction products.

2.5 The document D1 thus discloses all the technical features of the method claimed in independent claim 33 and dependent claims 34-35,37,45,48,51,52, and 54, as well as all technical features of the independent claim 55 related to a kit, since all the technical features of said kit are used in said method (s. above and col. 8-14).

2.6 Document D3 discloses primers/probes comprising one strand of a RNA polymerase promoter sequence (p. 34-35). The primers are used in a method for RNA amplification. The disclosed primer modification is suitable to act as template portion as well as as enzyme acting portions (fig. 1, 9-11). The T7 promoter sequence, as can be seen on page 43 of D3 comprises two identical sequences (ATA/ATA) that are separated by at least one enzyme acting portion: CTC is the recognition site of the restriction endonuclease Hind I. The primers/probes of D3 thus contain all technical features of the probes of claim 1.

2.7 The primers/probes of D3, that fall under the scope of claim 1 are used in a method that also involves the use of helper probes/primers (page 15, l. 15-19), the use of restriction enzymes (page 15), the use of a RNA polymerase (p. 28) implying the use of NTPs, the use of RNAseH (p. 28), the use of a DNA polymerase (p. 29) implying the use of dNTPs, buffers (p. 42) and ethidium bromide that can be regarded as detection substrate (p. 42). Consequently D3 discloses all technical features of claim 55.

2.8 In the light of D1 and D3 the subject matter of claims 1,2,5-9,24-25,33-35,37,45,48,51,52,54 and 55 is not novel. Consequently said claims do not fulfil the requirements of novelty of Article 33(2) PCT.

3 **INVENTIVE STEP** (Art. 33(3) PCT)

3.1 The dependent claims 3,4,10-23,26-32,36,38-44,46 and 47 do not seem to contain subject matter that could lead to an inventive claim. The subject matter of said claims merely seems to represents conventional features of standard probes or detection methods that are well known to the person skilled in the art. The use of DNAzymes for the detection of amplification products is well known and e.g. disclosed in D2. The person skilled in the art would use these alternatives and modifications without having to perform an inventive step.

3.2 In the light of D1 the subject matter of claims 1- 57 is not inventive and does not fulfil the requirements of inventive step of Article 33(3) PCT.

Claims

What is claimed is:

1. A probe molecule comprising single stranded or partially double stranded nucleic acid, wherein said probe comprising: target complementary portion, template portion, at least one enzyme acting portion, with or without 3' end block portion.

2. A probe according to claim 1, wherein said single stranded or partially double stranded nucleic acid is linear molecule.

3. A probe according to claim 1, wherein said single stranded or partially double stranded nucleic acid is circular molecule.

4. A probe according to claim 1, wherein said enzyme acting portions comprise a RNA polymerase promoter.

5. A probe according to claim 1, wherein said enzyme acting portions comprise RNase H acting sequences.

6. A probe according to claim 1, wherein said enzyme acting portions comprise a nuclease digestion site, wherein said nuclease digestion site support digesting opposite strand of said probe when double stranded.

7. A probe according to claim 6, wherein said enzyme acting portions comprise the combination of the RNase H acting sequences and the RNA polymerase promoter or the combination of the RNase H acting sequences and said nuclease digestion sites or the combination of said nuclease digestion sites and the RNA polymerase promoter or the combination of more than one of said nuclease digestion sites.

8. A probe according to claim 6, wherein said nuclease digestion site comprises modified nucleotides, whereby said digestion site on the probe is resistant to nuclease cleavage and the opposite unmodified strand is sensitive to cleavage.

9. A probe according to claim 8, wherein said modified nucleotides comprise phosphorothioate linkages,

10. A probe according to claim 6, wherein said nuclease digestion sites comprise restriction site having a restriction enzyme recognition sequence and a cleavage site.

11. A probe according to claim 10, wherein said restriction site comprises a type IIS restriction enzyme site.

12. A probe according to claim 11, wherein the enzyme cleavage site of said type IIS restriction site is located on target complementary portion.

13. A probe according to claim 12, wherein said type IIS restriction enzyme cleavage site corresponds to a SNP site, mutation nucleotide, methylation nucleotide or splicing site.

14. A probe according to claim 11, wherein said type IIS restriction site is the Fok I site.

15. A probe according to claim 1, comprising helper primer(s), wherein said helper primer comprises at least one portion complementary or substantially complementary to a part of said probe.

16. A probe according to claim 15, wherein said helper primer comprises a 3' end blocking moiety, whereby the 3' end of said helper primer is not extendible by a DNA polymerase.

17. A probe according to claim 15, wherein said helper primer does not comprise a 3' end blocking moiety, whereby the 3' end of said helper primer is extendible by a DNA polymerase.

18. A probe according to claim 15, wherein said helper primer comprises sequence complementary to the enzyme acting portion(s) with or without flanking sequences or to part of the enzyme acting portion(s) of said probe, whereby hybridization between said helper primer and said probe makes the enzyme acting portion(s) double stranded or partially double stranded.

19. A probe according to claim 15, wherein said helper primer comprises 3' end sequence complementary to a sequence 3' to one of the enzyme acting portions of said probe.

20. A probe according to claim 15, wherein said helper primer further comprises target complementary portion(s), wherein the target region(s) complementary to said helper primer is adjacent or substantially adjacent to the target region complementary to said probe.

21. A probe according to claim 20, wherein said helper primer comprises 3' and 5' target complementary portions, wherein the target region complementary to said probe is located in the middle of the target regions complementary to said helper primer and is adjacent or substantially adjacent to the target regions complementary to said helper primer.

22. A probe according to claim 1, wherein said target complementary portion comprises sequence complementary or substantially complementary to a target region of interest, whereby said target complementary portion of said probe hybridizes to said target region of interest and becomes double stranded, whereby one or more than one or part of the enzyme acting portion(s) of said probe is partially or fully functional.

23. A probe according to claim 1, wherein said enzyme acting portion(s), said target complementary portion and said template portion(s) of said probe overlap each other or have one portion embedded in other portions.

24. A probe according to claim 1, wherein said target complementary portion and/or said enzyme acting portion(s) and/or said template portion(s) of said probe comprise modified nucleotides, whereby modified nucleotides are resistant to nuclease cleavage.

25. A probe according to claim 1, wherein said target complementary portion and/or said enzyme acting portion(s) and/or said template portion(s) of said probe comprise chimeric RNA and DNA.

26. A probe according to claim 1, wherein said template portions comprise two identical or nearly identical sequences, which are separated by at least one enzyme acting portion.

27. A probe according to claim 26, wherein said at lease one enzyme acting portion comprises RNA polymerase promoter.

28. A probe according to claim 26, wherein said at lease one enzyme acting portion comprises restriction enzyme site.

29. A probe according to claim 3, wherein said probe is circular probe, wherein said circular probe comprises one template portion.

30. A probe according to claim 1, wherein said probe comprises a catalytically inactive antisense sequence complementary to a DNA enzyme in any place of the circular probe or within the 5' template portion with or without surrounding portion sequences of the linear probe.

31. A probe according to claim 30, wherein said DNA enzyme is 10-23 DNAzyme.

32. A probe according to claim 30, wherein said DNA enzyme is 8-17 DNAzyme.

33. A probe according to claim 1, wherein said 3' end block portion is chemical moiety, whereby 3' end of the probe is not extendible by a DNA polymerase.

34. A probe according to claim 1, wherein any end of said probe and/or helper primer is attached on a solid support.

35. A method of detecting a target nucleic acid sequence or multiple target nucleic acid sequences of interest in a sample, the method comprising the steps of:

(a) contacting probes or a set of probes in accordance with any one of the preceding claims with a nucleic acid sample under suitable hybridization conditions, wherein the target complementary portions of said probes or the target complementary portions of both said probes and helper primers hybridize the target sequence(s) and become double stranded, whereby one or more than one or part of the enzyme acting portion(s) of said probe is partially or fully functional;

(b) causing all enzyme acting portions of said probes double stranded and fully functional;

(c) treating said probes containing double stranded enzyme acting portion(s) so as to produce the single stranded end product (SSEP);

(d) annealing said SSEP to free probes and causing all enzyme acting portions of said probes double stranded and fully functional;

(e) repeating steps (c) and (d), whereby said probes are converted to double stranded or partially double stranded form, and multiple copies of said SSEP are produced repeatedly; and

(f) detecting directly or indirectly the end products so produced: double stranded end product, SSEP and pyrophosphate (PPi).

36. A method according to claim 35, wherein said method is performed in a single reaction or in separated reactions.

37. A method according to claim 35, wherein said target nucleic acid is RNA and said step (a) causes one of the enzyme acting portion, the RNase H digesting sites, double stranded and functional; wherein said step (b) comprises: digesting RNA strand by RNase H, extending the 3' end of partially digested strand using said probe as template by a DNA polymerase, whereby all other enzyme acting portions on said probes become double stranded and functional.

38. A method according to claim 37, wherein said extending the 3' end of partially digested strand further comprises strand displacing by said DNA polymerase or other strand displacement factors.

39. A method according to claim 37, wherein said other enzyme acting portions on said probes comprise restriction site or RNA polymerase promoter or both restriction site and RNA polymerase promoter.

40. A method according to claim 35, wherein one of said enzyme acting portions is restriction site and is located on the target complementary portion of said probe, said step (a) causes said restriction site double stranded and fully functional, wherein said step (b) comprises: digesting opposite strand of said probes on said restriction site by a restriction enzyme, and extending the 3' end of the digested strand using said probe as template by a DNA polymerase, whereby all other enzyme acting portions on said probes become double stranded and functional.

41. A method according to claim 40, wherein said extending the 3' end of the digested strand further comprises strand displacing by said DNA polymerase or other strand displacement factors.

42. A method according to claim 40, wherein said other enzyme acting portions on said probes comprise restriction site or RNA polymerase promoter or both restriction site and RNA polymerase promoter.

43. A method according to claim 40, wherein said restriction site is the only enzyme acting portion on said probe.

44. A method according to claim 35, wherein one of said enzyme acting portions is type IIS restriction site, wherein the cleavage site of said type IIS restriction site is located on target complementary portion of said probe and the recognition site of said type IIS restriction site is on either side of target complementary portion of said probe; wherein step (a) causes the target complementary portions of said probe double stranded, whereby a functional cleavage site of said type IIS restriction site is formed; wherein said step (b) comprises: annealing helper primers to said probes and causing said recognition sequence of said type IIS restriction site double stranded.

45. A method according to claim 44, wherein said annealing helper primers to said probes and causing said recognition sequence of said type IIS restriction site double stranded comprises: annealing said helper primers directly to said type IIS restriction enzyme recognition sequence with or without flanking sequences whereby double stranded recognition sequence of said type IIS restriction site is formed.

46. A method according to claim 44, wherein said annealing helper primers to said probes and causing said recognition sequence of said type IIS restriction site double stranded comprises: annealing the 3' end sequence of said helper primer to a sequence 3' to said type IIS restriction recognition sequence and extending the 3' end sequence of said helper primer by a DNA polymerase using said probe as template, whereby double stranded recognition sequence of said type IIS restriction site is formed.

47. A method according to claim 35, wherein in said step (a) the target complementary portions of said probes hybridize to free 3' end(s) of the target sequence(s), said step (b) comprises: extending said free 3' end(s) of the target sequence(s) by a DNA polymerase using said probes as templates, whereby other enzyme acting portions on said probes become double stranded and functional.

48. A method according to claim 35, wherein said enzyme acting portions of said probe comprise a restriction site, said step (c) comprises: digesting opposite strands of said probes on said restriction site by a restriction enzyme, extending the 3' end of the digested strand by a DNA polymerase, and repeating said digesting and said extending, whereby multiple copies of SSEP DNA are produced.

49. A method according to claim 48, wherein said extending the 3' end of the digested strand further comprises strand displacing by said DNA polymerase or other strand displacement factors.

50. A method according to claim 35, wherein said enzyme acting portions of said probe comprise RNA polymerase promoter, said step (c) comprises: repeated transcription by the RNA polymerase acting on said RNA polymerase promoter, whereby multiple copies of SSEP RNA are produced.

51. A method according to claim 35, wherein said enzyme acting portions of said probe comprise both restriction site and RNA polymerase promoter, said step (c) comprises: digesting opposite strands of said probes on said restriction site by a restriction enzyme, extending the 3' end of digested strands by a DNA polymerase, repeating said digesting and said extending, whereby multiple copies of SSEP DNA are produced, and repeated transcription by the RNA polymerase acting on said RNA polymerase promoter, whereby multiple copies of SSEP RNA are produced.

52. A method according to claim 51, wherein said extending the 3' end of the digested strand further comprises strand displacing by said DNA polymerase or other strand displacement factors

53. A method according to claim 35, wherein said SSEP are DNA molecules or RNA molecules or both DNA and RNA molecules, said step (d) comprises: annealing said SSEP to sequence portions of free probes and extending the 3' ends of said SSEP using said free probes as templates, whereby all enzyme acting portions of said probes become double stranded and functional.

54. A method according to claim 35, wherein said SSEP are RNA molecules, said step (d) comprises: annealing said SSEP to sequence portions of free probes, digesting said SSEP by RNase H, and extending the 3' end of partially digested SSEP using said free probes as templates, whereby all enzyme acting portions become double stranded and functional.

55. A method according to claim 35, wherein said probes are circular molecules, the sequences of said SSEP comprise one or more than one sequence unit that is complementary to said probes, step (d) comprises: annealing said SSEP to the whole or parts of said free probes, whereby said enzyme acting portions become double stranded and functional.

56. A method according to claim 35, wherein said template portions comprise antisense DNA enzyme, said method produces multiple copies of single stranded functional sense DNA enzyme, said step (f) of detecting single stranded end product comprises: including a RNA or DNA-RNA chimeric reporter substrate in the reaction, wherein said RNA or DNA-RNA chimeric reporter substrate comprises fluorescence resonance energy transfer fluorophores incorporated on either side of a DNAzyme cleavage site, cleaving said reporter substrate by sense DNA enzyme, whereby cleavage of said reporter substrate produces an increase in fluorescence signal.

57. A kit for use in detecting a target nucleic acid sequence or multiple target nucleic acid sequences of interest in a sample, said kit comprising: said a set or sets of probes, said helper primers, said detection substrate, said restriction enzymes, said RNA polymerase, said RNase H, said DNA polymerase, buffers, dNTPs, NTPs.